Induction of NANOG expression by targeting promoter sequence with small activating RNA antagonizes retinoic acid-induced differentiation.

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Public Summary:
RNA activation (RNAa) is a mechanism by which small double-stranded RNA (dsRNA) - termed small activating RNA (saRNA) - target gene regulatory sequences known as promoter to induce gene expression. This technique represents a novel approach to gene overexpression without the use of exogenous DNA. In the present study, we investigate whether RNAa can modulate expression of stem cell-related gene NANOG and manipulate cell fate. We screened and identified several saRNAs on NANOG promoter that can stimulate NANOG expression in human NCCIT embryonic carcinoma cells. NANOG induction by saRNA predictably modulates the expression of several known NANOG downstream target genes including FOXH1, REST, OCT4, and REX1. Treatment with a chemical compound retinoic acid (RA) triggers NCCIT cell differentiation reducing NANOG and OCT4 expression and upregulating several neural markers (i.e. ASCL1, NEUROD1, and PAX6). However, co-treatment with saRNA antagonizes NANOG downregulation and RA-induced differentiation. Overexpression of NANOG using a traditional vector-based method recapitulates saRNA results. Data from this study provides proof-of-concept that RNAa may be utilized to activate stem cell related genes and manipulate cell fate.

Scientific Abstract:
RNA activation (RNAa) is a mechanism by which small double-stranded RNA (dsRNA) - termed small activating RNA (saRNA) - target promoter sequences to induce gene expression. This technique represents a novel approach to gene overexpression without the use of exogenous DNA. In the present study, we investigate whether RNAa can modulate expression of the development-related gene NANOG and manipulate cell fate. Using a lentivirus-based reporter system as a screening tool, we identify synthetic saRNAs that stimulate NANOG expression in human NCCIT embryonic carcinoma cells. Mismatch mutations to saRNA duplexes define sequence requirement for gene activation. Functional analysis of NANOG induction reveals saRNA treatment predictably modulates the expression of several known downstream target genes including FOXH1, REST, OCT4, and REX1. Treatment with retinoic acid (RA) triggers NCCIT cell differentiation reducing NANOG and OCT4 expression and upregulating several neural markers (i.e. ASCL1, NEUROD1, and PAX6). However, co-treatment with saRNA antagonizes NANOG downregulation and RA-induced differentiation. Ectopic overexpression of NANOG via lentiviral transduction further recapitulates saRNA results providing proof-of-concept that RNAa may be utilized to activate development-related genes and manipulate cell fate.